Muscle contraction increases lactate transport while reducing sarcolemmal MCT4, but not MCT1

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Tonouchi, Mio, Hideo Hatta, and Arend Bonen. Muscle contraction increases lactate transport while reducing sarcolemmal MCT4, but not MCT1. Am J Physiol Endocrinol Metab 282: E1062–E1069, 2002—Rates of lactate uptake into giant sarcolemmal vesicles were determined in vesicles collected from rat muscles at rest and immediately after 10 min of intense muscle contraction. This contraction period reduced muscle glycogen rapidly by 37–82% in all muscles examined (P < 0.05) except the soleus muscle (no change P > 0.05). At an external lactate concentration of 1 mM lactate, uptake into giant sarcolemmal vesicles was not altered (P > 0.05), whereas at an external lactate concentration of 20 mM, the rate of lactate uptake was increased by 64% (P < 0.05). Concomitantly, the plasma membrane content of monocarboxylate transporter (MCT)1 was reduced slightly (P < 0.05), whereas at an external lactate concentration of 20 mM, MCT1 expression is chronically increased, either by training (2, 8, 38, 41, 44, 46) or by chronic electrical stimulation (7–21 days) (12, 38, 39). When muscle activity is decreased by denervation (37, 45, 53) or hindlimb suspension (19), lactate transport is decreased. However, in these models of chronically altered muscle activity, the changes in lactate transport have been associated with concurrent changes in the expression of muscle MCT1 and/or MCT4 proteins (2, 8, 21, 44, 53). It has been difficult to establish the relative contributions of MCT1 and MCT4 to these changes in muscle lactate transport. However, we have shown that lactate transport into muscle can be increased when only MCT1, but not MCT4, is increased (12).

Whether lactate flux across the plasma membrane is altered by an acute bout of exercise or muscle contraction is controversial. During contraction of perfused rat muscles, lactate influx, as measured by a dual tracer technique, was not altered (52). In isolated muscles we have observed a small (~10%) increase in lactate uptake after treadmill exercise (7) or no increase in lactate transport; GLUT4; giant vesicles

LACTIC ACID is produced by many tissues. Skeletal muscle is not only the key site of production of this metabolite, but muscle can also metabolize this substrate (4, 9, 23). Because of its low dissociation constant (pKₐ = 3.86), lactic acid is almost fully dissociated under physiological conditions. This is an impediment to its movement out of the muscle cell, because the lactate anion moves relatively slowly across the plasma membrane (29, 49). Therefore, lactate flux across the plasma membrane occurs via a facilitated diffusion mechanism that functions as a lactate-proton cotransport system (for review see Refs. 24, 29, and 49). In the past few years, a family of monocarboxylate transporters (MCTs) have been identified, which can facilitate the flux of lactate, as well as other monocarboxylates, across the plasma membrane (for review see Refs. 24 and 30).

In rat and human skeletal muscle, two MCT isoforms, MCT1 and MCT4, are expressed (11, 12, 15, 39, 40, 44, 47, 53). MCT1 expression is highly correlated with indexes of oxidative metabolism of muscle (40), whereas MCT4 expression is correlated with indexes of glycolytic metabolism in this tissue (11). In addition, the subcellular distribution of MCT1 and MCT4 in muscle differs. Both MCT1 and MCT4 are located in the plasma membrane and transverse tubules (11), and MCT1 is also found in the mitochondrial membrane (15), whereas MCT4, but not MCT1, is also present in an intracellular (endosomal) pool (11). This may indicate that lactate taken up into the muscle cell can also be readily taken into the mitochondria via MCT1, whereas the intracellular pool of MCT4 may represent a reservoir of transporters that may possibly be translocated to the plasma membrane with the appropriate stimulus, such as muscle contraction.

A number of studies have shown that skeletal muscle lactate transport can be increased when muscle activity is chronically increased, either by training (2, 8, 38, 41, 44, 46) or by chronic electrical stimulation (7–21 days) (12, 38, 39). When muscle activity is decreased by denervation (37, 45, 53) or hindlimb suspension (19), lactate transport is decreased. However, in these models of chronically altered muscle activity, the changes in lactate transport have been associated with concurrent changes in the expression of muscle MCT1 and/or MCT4 proteins (2, 8, 21, 44, 53). It has been difficult to establish the relative contributions of MCT1 and MCT4 to these changes in muscle lactate transport. However, we have shown that lactate transport into muscle can be increased when only MCT1, but not MCT4, is increased (12).

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tate uptake after 30 min of low-intensity electrical stimulation of the sciatic nerve (43). However, recently it was observed that lactate uptake by small sarcolemmal vesicles (~0.5 μm diameter) is reduced after submaximal (~24%) and exhaustive exercise (~57%). However, these reductions occurred only at low concentrations of external lactate (1 mM) (18, 20), not at higher external lactate concentrations (5–75 mM lactate), after either submaximal (20) or exhaustive exercise (18). In contrast, at extremely high external lactate concentrations (100 mM), lactate uptake was markedly increased (+112%), but only after exhaustive exercise (18), not after submaximal exercise (20). Thus recent studies suggest that lactate transport may be altered by acute exercise, although for unknown reasons this may depend on the lactate concentration used in the transport studies, and possibly the exercise intensity.

Lactate transport studies with intact muscles are problematical because of the concurrent lactate metabolism. Studies with small vesicles (<1 μm) (1, 18, 20, 42) may also be problematical, because a large number of the vesicles are oriented wrong side out (~40%) (18, 20, 42). This may be especially critical if the inward and outward transport capacities of MCT1 and MCT4 differ, as is indirectly suggested by many studies (11, 12, 15, 39, 40, 44, 47, 53), although specific data are not yet available. It is known that the lactate transport kinetics of these two MCT isoforms also differ considerably. The Michaelis-Menten kinetic constant ($K_m$) for MCT1 is in the range of 3–5 mM (14, 36), and the $K_m$ for MCT4 is in the range of 20–30 mM (13, 17, 36). Comparisons among published studies indicate that, in mixed rat hindlimb muscles, the $K_m$ for lactate transport is in the range of 13–40 mM (29). However, despite dissimilar concentrations of MCT1 (red >> white muscle) and only slight differences in MCT4 in fast-twitch red and white skeletal muscles (~10%) (11, 12, 39, 40, 53), the $K_m$ for lactate transport in red and white muscles appears to be quite similar (~15 mM) (31). This $K_m$ is comparable to that reported for MCT4 (17, 36), although changes in only MCT1 expression are sufficient to increase lactate uptake by muscle (12). Examination of muscle contraction-induced changes in lactate transport, in relation to the $K_m$ of MCT1 and MCT4, have not yet been performed.

In the present study, we have examined MCT1, MCT4, and lactate transport in giant sarcolemmal vesicles after a brief (10-min) intense period of electrical stimulation. For these purposes, we examined uptake rates of lactate uptake at two concentrations, namely 1 and 20 mM lactate. These concentrations are near the $K_m$ values of MCT1 and MCT4, respectively (13, 14, 17, 36). Lactate uptake was determined in giant sarcolemmal vesicles. Unlike the small vesicle preparations, in which only 60–70% of the vesicles are oriented right side out (18, 20, 42), the giant vesicles are oriented 100% right side out (29, 48). Moreover, these giant vesicles have been used previously to examine contraction-induced glucose (48) and fatty acid transport (5).

**Methods**

**Animals.** Male Sprague-Dawley rats were used for this study. They were housed in an air-conditioned room on a 12:12-h light-dark cycle, with food and water available ad libitum. Ethical approval for this work was obtained from the animal care committee at the University of Waterloo.

**Electrical stimulation.** After rats had been anesthetized (Somnotol, 60 mg/kg ip), a small incision was made on the lateral side of one of the thighs to expose the sciatic nerve that innervates the hindlimb muscles. Stimulating electrodes were placed on the sciatic nerve that had been severed. Stimulus pulses (50–60 V, stimulation rate 100 Hz, train delay <0.01 ms, train duration 200 ms, pulse duration 100 μs) were given for 2 × 5 min interrupted by a 1-min rest interval. These stimulation rates are known to increase skeletal muscle glucose transport (26). After the stimulation, the hindlimb muscles were rapidly removed, and giant vesicles were prepared from fresh tissues. Vesicles from the contralateral, noncontracting muscles served as a control. In a separate group of animals, muscles were taken for analyses of muscle glycogen. In another group of animals, we perfused hindlimb muscles, as we have previously reported in detail (11, 12, 39, 40), and measured isometric force production induced by electrical stimulation, as we have reported (26). Briefly, for this purpose, the knee joint was fixed with a steel pin under the tibiopatellar ligament, and the Achilles tendon was connected to a calibrated force transducer (26). Force output was recorded on an eight-channel recorder. At the same time, we measured lactate (Stat Profile Plus 9, Nova Biomedical Canada, Mississauga, ON, Canada) efflux from the muscle in the venous effluent. Perfusion samples were drawn just before the start of each 5-min contraction period and immediately at the end of this 5-min contraction period.

**Preparation of giant sarcolemmal vesicles.** Sarcolemmal vesicles were purified as we have previously described (5, 6). Briefly, muscles were scissored lengthwise into thin slices and incubated in 140 mM KCl, 5 mM MOPS, pH 7.4, 150 U/ml collagenase (Sigma type VII), and 0.01 mg/ml of the protease inhibitor aprotinin (Sigma, A1153) for 1 h at 34°C. A three-layer-step-density gradient was used to isolate the vesicles. The upper layer was composed of KCl-MOPS (3 ml), the middle layer was composed of 4% Nicodenz in KCl-MOPS (3 ml), and the bottom layer contained the vesicle suspension (8 ml). The vesicles were removed from the interface of the two upper layers after centrifugation (60 g for 45 min) at room temperature. Thereafter, the vesicles were diluted with KCl-MOPS and recovered with a final centrifugation step (12,000 g for 4 min).

**Lactate uptake.** Lactate uptake measurements were performed under zero-trans conditions in giant vesicles (50–80 μg protein) at concentrations of 1 and 20 mM lactate (0.1 μCi [U-14C]lactate/tube). For these purposes, lactate was added to the vesicle suspension and vortexed. Preliminary studies demonstrated that lactate uptake increased linearly up to 30 s. Therefore, in the present studies we used a 10-s uptake period. Uptake was terminated by the addition of an ice-cold stop solution (3 mM HgCl in 0.1% BSA, KCl-MOPS). The vesicles were then centrifuged (12,000 g for 4 min), and the supernatant fraction was discarded. To determine nonspecific 14C associated with vesicles, the stop solution was added to the vesicles before the lactate solution was loaded. 14C activity was determined with a liquid scintillation counter.

**Detection of MCT1, MCT4, and GLUT4.** Aliquots of the same giant vesicles that were used in the lactate transport assays were used to detect MCT1 and MCT4 with Western blotting, as we have previously described in detail (5, 11, 12,
In some additional experiments, we also measured the contraction-induced changes in plasma membrane GLUT4, MCT1, and MCT4 from the same samples. Finally, we also examined the MCT1 and MCT4 content in the plasma membranes of giant vesicles and the vesicular cytosol. For these purposes vesicles were ruptured by three freeze-thawing cycles in liquid nitrogen, and then the plasma membranes were separated from the cytosol using high-speed ultracentrifugation (100,000 g for 60 min). MCT1, MCT4, and GLUT4 protein band densities were quantified by scanning the blots on a densitometer connected to a computer with appropriate software.

Muscle glycogen. For glycogen determination, a small portion of powdered muscle (10 mg) was extracted in 0.1 M NaOH and neutralized with 0.1 M HCl. After the samples were freeze-dried using standard procedures, glycogen was assayed as described by Harris et al. (27).

RESULTS

Muscle force, lactate production, and glycogen reduction in electrically stimulated muscle. Muscle force (Fig. 1A) was sharply reduced during the first 5 min of contraction (−93%; $P < 0.05$). After a 1-min rest period, at the start of the second 5-min contraction bout, muscle force had recovered somewhat, to 30% of the initial force production ($P < 0.05$). This declined again during the next 5 min of contraction to 5% of initial force production ($P < 0.05$).

Lactate efflux (Fig. 1B) in the first exercise bout was increased sixfold ($P < 0.05$). By the end of the second 5-min bout, lactate production was still threefold greater than at rest ($P < 0.05$), although it was 47% less than at the end of the first 5-min contraction period ($P < 0.05$).

Glycogen concentrations were significantly decreased in red gastrocnemius (RG), white gastrocnemius (WG), extensor digitorum longus (EDL), red tibialis anterior (RTA), and white tibialis anterior (WTA) muscles ($P < 0.05$) by 2 × 5 min of intense muscle contraction (Fig. 1C). The decrease in glycogen in white muscle (WG and WTA) was significantly greater (−74.7% and −81.6%, respectively) than in red muscle (RG and RTA, −36.7% and −47%, respectively) ($P < 0.05$). No changes in soleus (SOL) muscle glycogen were observed ($P > 0.05$).
Lactate uptake by giant sarcolemmal vesicles. Muscle contraction did not alter vesicular lactate uptake at a low (1.0 mM) external lactate concentration (Fig. 2, \( P > 0.05 \)). In contrast, vesicular lactate uptake was increased 64\% by muscle contraction when vesicles were incubated with a high (20 mM) external lactate concentration (Fig. 2, \( P < 0.05 \)).

Sarcolemmal MCT1, MCT4, and GLUT4. Muscle contraction caused a very small (10\%) reduction in MCT1. This small decrease was, however, statistically significant (Fig. 3, \( P < 0.05 \)). In the same vesicles, sarcolemmal MCT4 content was decreased by 25\% (Fig. 3, \( P < 0.05 \)). The decrease in MCT4 was greater than that of MCT1 (\( P < 0.05 \)).

As a positive control, we also determined whether we could detect contraction-induced increases in sarcolemmal GLUT4 content. Therefore, we conducted additional experiments using the same muscle contraction and vesicle preparation protocols. In the same vesicles, sarcolemmal GLUT4 was increased (Fig. 4, \( P < 0.05 \)), whereas no changes were detected in MCT1 (Fig. 4, \( P > 0.05 \)), and there was again a reduction (\(-20\%\)) in MCT4 (Fig. 4, \( P < 0.05 \)).

To ascertain whether the electrical stimulation caused some of the MCT1 or MCT4 to detach from the plasma membrane and be relocated to the cytosol, these MCTs were also measured in the cytosol and the plasma membrane separately. This work demonstrated that neither MCT1 nor MCT4 was present in the cytosol compartment of the vesicles, whereas the reductions in plasma membrane MCT4 are clearly evident (Fig. 5).

**DISCUSSION**

We have examined the effects of an acute (10-min) bout of muscle contraction on vesicular lactate uptake at low (1.0 mM) and high external lactate concentrations (20 mM). In addition, we examined in the same vesicles the plasma membrane content of MCT1 and MCT4. There are several novel observations. We have found that muscle contraction 1) increases lactate uptake, but only when external lactate concentrations are high (20 mM), and that this occurs 2) in the face of very small or no changes in sarcolemmal MCT1 and 3) a decreasing sarcolemmal content of MCT4.

**Fig. 2.** Lactate uptake by giant sarcolemmal vesicles (means ± SE). At 1 mM external lactate concentrations, \( n = 12 \) control and \( n = 12 \) stimulated vesicle preparations. At 20 mM external lactate concentrations, \( n = 16 \) control and \( n = 16 \) stimulated vesicle preparations. *\( P < 0.05 \), control vs. stimulated muscle.

**Fig. 3.** Representative giant vesicle monocarboxylate transporter (MCT1 and MCT4) blots in control and electrically stimulated muscles (A), and quantification of giant vesicle MCT1 and MCT4 from control and electrically stimulated muscles (B). Values are means ± SE; \( n = 13 \) control and \( n = 13 \) stimulated vesicle preparations. MCT1 and MCT4 were determined from the same vesicle preparations. *\( P < 0.05 \), control vs. stimulated, **\( P < 0.05 \), MCT1 vs. MCT4 in electrically stimulated muscles.
It should be noted that, in an effort to describe the lactate production and intensity of the contractile activity (i.e., a \(2/5\) min contraction period interspersed with 1 min of rest), a somewhat different procedure was required than that used in the experiments in which we prepared giant vesicles. In these latter experiments, we used a \(2/5\) min isotonic contraction procedure (i.e., all the data shown in Fig. 1C through Fig. 5), but we found that it was necessary to use a perfused hindlimb preparation and a \(2 \times 5\) min isometric contraction period to ascertain muscle lactate efflux and muscle force production (i.e., data shown in Fig. 1, A and B). Although these protocols are not directly comparable, both approaches demonstrated qualitatively that the \(2 \times 5\) min contraction period represented a very intense bout of exercise. This can be seen by the rapid and large changes in lactate efflux and force production in perfused, isometrically contracting muscles (Fig. 1, A and B) and the large decrease in muscle glycogen in isotonically contracting muscles (Fig. 1C).

Previous studies from our laboratory that have examined skeletal muscle lactate uptake immediately after muscle contraction have observed only modest increases (\(\sim 10\%\)) after treadmill running (7) or no change after 30 min of electrical stimulation of the sciatic nerve (43). These studies, however, were conducted in isolated skeletal muscles in which, for technical reasons, the lactate uptake measurements occurred 30–60 min after exercise while muscles remained metabolically active in vitro. It is possible that if the exercise-induced changes in lactate uptake are transient, then any effects of exercise could have been missed in these studies. A similar concern would also apply to the present studies, because there is also a time delay between the end of muscle contraction and the measurement of lactate uptake because of the preparation time required to obtain the giant vesicles.

Lactate uptake determinations have been performed after exercise, either by use of small vesicles (<1 \(\mu\)m diameter), of which a large number (\(\sim 40\%\)) are oriented wrong side out (18, 20), or giant sarcolemmal vesicles (15 \(\mu\)m diameter, present study), which are oriented 100% right side out (29). In our present studies, we did not observe any changes in lactate uptake after 10 min of intense muscle contraction at low external lactate concentrations (1 mM). This differs from the results of studies by Eydoux and colleagues (18, 20, 21), in which there was a decrease in lactate uptake after submaximal and exhaustive treadmill exercise, when the external lactate concentration in the assay was low (1.0 mM) (18, 20, 21). The increase in lactate uptake induced by 10 min of muscle contraction in the present study, at a lactate concentration of 20 mM, has not been observed by others, either after exhaustive exercise (18, 21) or after submaximal treadmill exercise (20). These investigators have reported that lactate uptake after submaximal or maximal exercise is not altered at lactate concentrations of \(5–75\) mM. However, at a very high lactate concentration (100 mM), these investigators have at times observed an increase in lactate uptake with maximal exercise (18, 21) but not with submaximal exercise (20). These differences cannot be explained by differences in glycogen loss induced by exhaustive and submaximal exercise, because these decrements were quite similar in their...
studies (18, 20). It was suggested that the increase in lactate uptake at 100 mM lactate might be due to the altered diffusional capacity of the muscle (18), although no data were presented to substantiate this suggestion. Whether the marked discrepancies in contraction-induced lactate uptake in our studies and those of others (18, 20) are due to the different vesicle preparations used is not known. Certainly, the altered plasma membrane orientation (~40% wrong side out in small vesicles) is a concern in some studies (18, 20), if there are different inward and outward lactate transport rates associated with MCT1 and MCT4.

To date, there have been no studies in which the plasma membrane content of MCTs has been examined after a bout of muscle contraction or exercise. In the present study, we observed only a very small change (−10%) in MCT1 in one set of studies, whereas in a second set of studies there was no change in MCT1. In a recent study, we also observed that a more modest rate of muscle contraction (40 tetani/min for 30 min) did not alter sarcolemmal MCT1 (5). Thus, on the basis of these studies, it appears that there is essentially no change in the concentration of sarcolemmal MCT1 with muscle contraction. This is not surprising, since MCT1 is not present in an intracellular pool (11), whereas it may be present in the mitochondria (15). However, this organelle is not presumed to be a compartment from which transport proteins are cycled to the plasma membrane.

In the two sets of experiments performed in this report, we found that MCT4 was consistently reduced by 20–25% after 10 min of intense muscle contraction. Whether muscle contraction causes this transporter to recycle to its intracellular compartment (11) is not known. Moreover, it is not clear why the surface MCT4 is reduced. However, it is noteworthy that this is not an artifact, because in the same vesicles in which MCT4 was reduced, MCT1 was not altered, and the well known contraction-induced increase in GLUT4 was observed (see Fig. 4). Moreover, we could not detect MCT4 in the cytosol of the giant vesicles, indicating that MCT4 was not simply present in the cytosol near the plasma membrane.

Because we had previously (11) identified an intracellular compartment of MCT4, we expected that any changes in lactate transport might be due to an increased plasma membrane content of MCT4, assuming that the intracellular MCT4 could be translocated to the plasma membrane by muscle contraction. However, the results of the present study have shown that the relationship between MCT transporters and lactate transport appears to be more complex than has been assumed. On the basis of the kinetics of MCT1 ($K_m = 3–5$ mM) (14, 36), one could speculate that the lack of change in lactate uptake, when lactate in the assay is low (1.0 mM), is due to the unaltered sarcolemmal MCT1. We have assumed that changes in lactate transport at 20 mM are most closely associated with MCT4 ($K_m = 20–30$ mM (17, 36)]. However, the decrease in MCT4 and the concurrent increase in lactate transport at 20 mM lactate are surprising. It is possible that there may also be another MCT in muscle. In hamster, MCT2 is present in muscle (28), although it does not appear to be present in rat muscle. However, in other studies, we have observed that MCT2 mRNA and protein are detectable in muscle (A. Bonen, unpublished data). However, MCT2 appears to be a very high-affinity ($K_m = 25–50$ μM) pyruvate transporter (13, 34).

Because we cannot account for the increased rate of lactate transport at high external lactate concentrations (20 mM) by an increase in plasma membrane MCT4, or for that matter by MCT1, it appears that either an increase in diffusion or an increased intrinsic activity of the MCTs provides possible explanations for the increased lactate uptake. Changes in sarcolemmal diffusional capacity have been measured after 10 wk of myocardial volume overload in muscle (1). However, in the present study, changes in diffusional capacity would seem to be very unlikely after only 2 × 5 min of contractile activity interspersed by a 1-min rest period. Moreover, diffusional uptake of glucose (32) or fatty acids (5) after exercise or electrical stimulation is not altered. On the other hand, it has been shown that the activities of glucose transporters GLUT4 and GLUT1 (3, 10, 16, 22, 25, 33, 50, 51), as well as the activity of the fatty acid transporter FAT/CD36 (35), can be altered. Thus we speculate that the intrinsic activity of one or both of the MCTs was altered to increase lactate transport.

In summary, we have shown that muscle contraction increases lactate uptake but that this is observed only at high concentrations (20 mM), and not at low concentrations (1 mM), of external lactate. We suggest, on the basis of the lactate transport kinetics of MCT1, that the small change or lack of change in sarcolemmal MCT1 in contracting muscle may account for the lack of change in lactate uptake at low external lactate concentrations. However, it is difficult to associate the increase in lactate uptake, in the presence of high external lactate concentrations, with the concomitantly decreased levels of sarcolemmal MCT4 or the unchanged levels of sarcolemmal MCT1. Therefore, it appears that the contraction-induced increase in lactate transport is attributable to changes in the intrinsic activity of the MCT transporters.

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